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The optimum ratio of standardized ileal digestible leucine to lysine for 8 to 12 kg female pigs¹

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ABSTRACT: The objective of the study was to estimate Leu requirement for weaned piglets to balance indispensable AA in reduced CP diets. A dose-response experiment was conducted to estimate the standardized ileal digestible (SID) Leu to Lys ratio required for the maximum growth of young pigs after weaning. In this study, 96 female pigs (initial BW of 8 kg) were allotted to 1 of 6 dietary treatments with 16 individually penned pigs per treatment. Graded levels of crystalline L-Leu were added to a basal diet to provide diets containing 0.70, 0.80, 0.90, 1.00, 1.10, and 1.20 SID Leu:Lys. Lysine was limiting and fulfilled 90% of the current recommendations. The ADFI, ADG, and G:F were determined during a 2 wk experimental period. Blood and urine samples were taken at the end of each wk. The ADFI increased linearly ($P < 0.001$) from 0.70 to 0.80 SID Leu:Lys and then remained constant from 0.90 to 1.20 SID Leu:Lys.

The ADG showed a quadratic increase ($P = 0.02$), as the SID Leu:Lys level increased from 0.70 to 0.90 SID Leu:Lys and did not change further from 0.90 to 1.20 SID Leu:Lys. The G:F increased quadratically ($P < 0.001$) with increasing SID Leu:Lys level, and the greatest G:F was achieved with pigs receiving the diet with 0.80 SID Leu:Lys. Increasing the dietary SID Leu:Lys resulted in a linear increase in plasma Leu concentration ($P < 0.001$) and quadratic increases ($P < 0.001$) in plasma Cys concentration. The plasma concentration of most of the other AA was lowest in pigs receiving the diets with 0.90 to 1.00 SID Leu:Lys. The plasma urea nitrogen concentration tended ($P = 0.08$) to be lowest in pigs receiving 1.00 SID Leu:Lys, suggesting a more balanced AA profile at this level. Using a curvilinear-plateau model, the SID Leu:Lys requirement was estimated at 0.93 to maximize growth in female pigs weighing 8 to 12 kg.

Keywords: dose-response, growth performance, leucine requirement, pigs

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INTRODUCTION

Generally, Leu is found in abundant amounts in common feed ingredients used in practical pig diets. Due to the important roles of Leu in regulating muscle protein synthesis and the activity of enzymes involved in oxidative decarboxylation of the branched-chain amino acids (BCAA), determination of the Leu requirement is of interest. Moreover, the balance of

BCAA is crucial to keep the blood-brain barrier function. Leucine is known to increase protein synthesis by activation of the mammalian target of rapamycin (mTOR) signaling pathway followed by upregulation of mRNA translation (Cynober and Harris, 2006). This effect originates from α -ketoisocaproic acid (KIC), the product of Leu transamination. Excess Leu could affect the requirement of the other 2 BCAA as it is stimulating the branched-chain keto acid dehydrogenase complex (BCKDH), which catalyzes an irreversible step of BCAA catabolism (Murakami et al., 2005; Wiltafsky et al., 2009). Also, some AA including His, Phe, Ile, Val, Trp, Tyr, Met, Thr, and Glu are transported across the blood-brain barrier by the same transporter as Leu (Smith, 2000). The greater affinity of the transporter for Leu (sevenfold greater) and

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Phe (2.5-fold) than Ile causes impaired transport of Ile when Leu is in excess (Smith, 2000). Considering these important roles of Leu and the interest to optimize low CP diets with improved AA profiles, as well as consequences of excess Leu, it is necessary to determine the requirements for all nondispensable AA, including Leu.

Only a few studies report the Leu requirements for piglets after weaning (Gatnau et al., 1995, Augspurger and Baker, 2004, Gloaguen et al., 2013a, 2013b). The current recommendations for Leu requirement for growth (relative to Lys) are 1.02 (Tybirk et al., 2012), 1.00 (NRC, 2012), 1.00 (BSAS, 2003), and 1.01 (Gloaguen et al., 2013b). This study aimed to determine the standardized ileal digestible (SID) Leu requirements in ratio to Lys in pigs from 8 kg.

MATERIALS AND METHODS

The experiment complied with the Danish Ministry of Justice, Law No. 253 of March 8, 2013, concerning experiments with animals and care of experimental animals and a license issued by the Danish Animal Experiments Inspectorate.

Animals and Diets

A total of 96 crossbred (Danish Landrace, Yorkshire × Duroc) female pigs were individually housed in 1 × 2.2 m pens with one-third concrete floor and two-thirds cast iron slatted floor that were placed in 4 identical rooms. The temperature and humidity were kept around 22°C and 60%, respectively, during the experimental period.

Diets were based on wheat, barley, and soy protein concentrate and were formulated to have the same CP (154 g/kg diet) and net energy content (10.4 MJ/kg; Table 1). The ingredients and experimental diets were analyzed before the experiment to confirm a correct mixing of diets. The diets were planned to contain 0.70, 0.80, 0.90, 1.00, 1.10, and 1.20 of SID Leu:Lys, with Lys being limiting. Diets provided 11.8 g/kg total Lys or 11.0 g SID Lys/kg corresponding to 90% of the Lys requirement (Tybirk et al., 2012). The other nondispensable AA and calcium and phosphorus were supplied according to or slightly exceeding the Danish recommendations for pigs weighing 9–15 kg (Tybirk et al., 2012). The analyzed and calculated compositions of the experimental diets are presented in Table 2.

Experimental Design

The experiment was conducted in 2 replicates with a duration of 2 wk each. Pigs were weaned at 28 d of age, and the experiments started 1 wk after weaning with an average initial BW of 7.8 ± 0.9 kg (mean ± SD).

Table 1. Composition of experimental diets (g/kg as-fed)

Item	Basal diet ¹
Wheat	726.1
Barley	100.0
Soy protein concentrate ²	78.9
Animal fat	20.0
Calcium carbonate	16.3
Monocalcium phosphate	14.8
NaCl	4.4
Vitamin-mineral premix ³	4.0
DL-Met (99%)	2.9
L-Glu (98%) ⁴	–
L-His (98%)	1.4
L-Ile (98%)	2.4
L-Lys HCl (78%)	8.5
L-Thr (98%)	4.0
L-Trp (40%)	1.3
L-Phe (98%)	3.3
L-Tyr (98%)	1.0
L-Val (99%)	3.8
Phytase ⁵	0.2
Microgrits ⁶	0.7

¹L-Leu (98%) added to the basal diet at graded levels of 0.0, 1.1, 2.2, 3.3, 4.5, and 5.6 g/kg to provide 0.70, 0.80, 0.90, 1.00, 1.10, and 1.20 SID Leu:Lys levels.

²HP300 (Hamlet Protein, Horsens, Denmark).

³Provided the following per kg of diet: 10,000 IU vitamin A, 2,000 IU vitamin D3, 94 IU vitamin E, 2.4 mg vitamin K3, 2.4 mg vitamin B1, 4.8 mg vitamin B2, 2.4 mg vitamin B6, 0.02 mg vitamin B12, 12 mg D-panthothenic acid, 26 mg niacin, 0.2 mg biotin, 200 mg Fe (Fe(II) sulfate), 165 mg Cu (Cu(II) sulfate), 200 mg Zn (Zn(II) oxide), 56 mg Mn (Mn(II) oxide), 0.3 mg KI, 0.3 mg Se (Se-selenite).

⁴Included at the basal diet at graded levels of 0.60, 0.49, 0.38, 0.27, 0.15, and 0.04 to compose isonitrogenic diets.

⁵Natuphos 5000, 5,641 FTU/g (BASF, Ludwigshafen, Germany).

⁶Corn bran in various colors to identify diets.

To determine the minimum SID Leu:Lys supply to maximize performance, a dose-response experiment was conducted. Pigs were allotted to 1 of the 6 experimental diets, had ad libitum access to feed and water, and were weighed on d 7 and 14 without a preceding fast. The ADFI and ADG were determined at the end of each wk.

At d 8 and 15 of the experiment, after an overnight fast, pigs were supplied with 25 g/kg BW^{0.75} of feed at 0700 h, and blood and urine samples were taken 3 h later from 8 pigs per treatment. Blood samples were collected by jugular vein puncture into 10 mL heparinized tubes (Greiner BioOne GmbH, Kremsmünster, Austria) and placed on ice after collection. Blood samples were centrifuged at 1,050 × g at 4°C for 10 min, and the plasma was immediately harvested and stored at –80°C until the laboratory analysis. Urine samples were collected using tampons covered by cotton pads in a way that keeps tampons away from fecal contamination. The sandwiched tampons were mounted on the back of the pigs with surgical tape at the time of blood collection until urination. The tampons were stored on ice immediately

Table 2. Calculated and analyzed composition of experimental diets with different Leu content (g/kg as-fed)¹

Item	SID ² Leu:Lys					
	0.70	0.80	0.90	1.00	1.10	1.20
Calculated composition						
Crude protein	154.0	154.0	154.0	154.0	154.0	154.0
Lys	11.8	11.8	11.8	11.8	11.8	11.8
Met	4.7	4.7	4.7	4.7	4.7	4.7
Met + Cys	7.3	7.3	7.3	7.3	7.3	7.3
Thr	8.2	8.2	8.2	8.2	8.2	8.2
Ile	7.1	7.1	7.1	7.1	7.1	7.1
Leu	9.0	10.1	11.2	12.2	13.3	14.4
Val	9.5	9.5	9.5	9.5	9.5	9.5
Phe	8.5	8.5	8.5	8.5	8.5	8.5
His	4.3	4.3	4.3	4.3	4.3	4.3
Analyzed composition						
Crude protein (N × 6.25)	157.3	155.8	153.8	158.3	155.1	158.5
Lys	11.77	11.77	10.72	11.89	11.36	11.60
Met	4.51	4.48	4.29	4.57	4.46	4.52
Met + Cys	7.20	7.17	6.98	7.28	7.14	7.22
Thr	7.90	8.00	7.73	8.07	7.90	7.99
Ile	7.01	7.06	6.80	7.07	6.95	7.11
Leu	8.87	9.93	10.72	12.28	13.01	14.27
Val	9.25	9.33	9.03	9.47	9.21	9.34
Phe	9.28	9.35	9.09	9.37	9.28	9.37
His	4.36	4.36	4.18	4.39	4.29	4.39
Ala	5.03	5.06	4.98	5.08	5.01	5.11
Arg	7.17	7.24	7.09	7.29	7.18	7.35
Asp	9.39	9.44	9.18	9.58	9.45	9.63
Glu	35.17	34.41	33.06	32.40	31.04	30.46
Gly	5.22	5.24	5.17	5.28	5.20	5.29
Pro	10.03	10.08	10.15	10.10	10.08	10.17
Ser	5.90	5.94	5.86	6.00	5.94	6.02

¹Three samples of each diet were analyzed.

²SID = standardized ileal digestible.

after urination and were centrifuged at $3,000 \times g$ at 4°C for 10 min for collection of urine. Pigs were monitored daily for health conditions and were treated with antibiotics (Oxytetracyclin, Engemycin; MSD Animal Health, Wellington, New Zealand) in the case of diarrhea.

Chemical Analysis

Nitrogen content was analyzed by a modified Kjeldahl method (AOAC, 2000), and CP content was estimated as total nitrogen $\times 6.25$. Representative samples ($n = 3$) of each diet were hydrolyzed for 23 h at 110°C with (for Cys and Met) or without (for all other AA) performic acid oxidation, and AA were separated by ion exchange chromatography and quantified by photometric detection after ninhydrin reaction (European Commission, 1998).

Plasma-free AA and urea were analyzed using an AA analyzer fitted to a lithium high-performance

system for physiological AA (Biochrome 30+ Amino Acid Analyzer; Biochrome, Cambridge, UK). The AA analyzer was calibrated using a standard for acidic, neutral, and basic AA (Sigma Aldrich, St. Louis, MO). Urine urea nitrogen and creatinine were determined according to standard procedures (Siemens Diagnostics Clinical Methods) by using an autoanalyzer, ADVIA 1650 Chemistry System (Siemens Medical Solutions, Tarrytown, NY).

Calculations and Statistical Analysis

The G:F was calculated as ADG divided by ADFI. The urea content of urine was divided by the creatinine content to provide the urea to creatinine ratio (Kaneko et al., 1998).

The data were analyzed by the MIXED procedure of SAS (Version 9.3; SAS Inst. Inc., Cary, NC). The experimental unit was the individual pig. The model included diet as fixed effect and room and period as random effects. For plasma AA and plasma urea nitrogen (PUN), the average of the 2 wk is presented, and the statistical analysis therefore included wk as fixed effect. Initial BW was included in the model as a covariate. Orthogonal polynomial contrast coefficients were used to determine linear and quadratic effects of increasing SID Leu:Lys on the measured traits. The PROC NLIN and NLMIXED procedures of SAS were used to estimate the optimum Leu:Lys for ADFI and ADG by subjecting the least squares means of the response traits to broken-line and curvilinear plateau models and G:F to curvilinear plateau models (Robbins et al., 2006). The broken-line model could not fit to the G:F data. The models are described in the figures for the response criteria. Statistical significance was accepted at $P < 0.05$ and tendencies at $P < 0.10$. Data are presented as least squares means and SEM.

RESULTS

Table 3 presents the data on initial and final body weights of pigs as well as the effects of increasing the SID Leu:Lys on ADFI, ADG, and G:F. The ADFI increased linearly ($P < 0.001$) from 0.70 to 0.80 SID Leu:Lys to reach a plateau thereafter when SID Leu:Lys was increased up to 1.20. Final body weight was lowest for pigs fed 0.70 SID Leu:Lys and was not different among the 0.80, 0.90, 1.00, 1.10, and 1.20 SID Leu:Lys diets. The ADG showed a quadratic increase ($P = 0.02$) as the SID Leu:Lys level increased from 0.70 to 0.90 and did not change from 0.90 to 1.20 SID Leu:Lys. The G:F increased quadratically ($P < 0.001$) by increasing the SID Leu:Lys level, and the greatest G:F was achieved for pigs fed 0.80 SID Leu:Lys.

Table 3. Effect of increasing the ratio of standardized ileal digestible (SID) Leu:Lys on performance of the pigs¹

Item	SID Leu:Lys						SEM	<i>P</i> -value ²		
	0.70	0.80	0.90	1.00	1.10	1.20		ANOVA	Linear	Quadratic
Initial BW, kg	7.77	7.69	7.78	7.79	7.79	7.82	0.68	–	–	–
Final BW, kg	10.76 ^a	11.74 ^b	12.11 ^b	12.17 ^b	12.18 ^b	12.27 ^b	0.32	0.001	0.001	0.02
ADFI d 0–14, g/d	359 ^a	399 ^{ab}	456 ^{bc}	456 ^{bc}	454 ^{bc}	479 ^c	25	0.002	< 0.001	0.13
ADG d 0–14, g/d	212 ^a	281 ^b	308 ^b	312 ^b	313 ^b	320 ^b	23	0.001	< 0.001	0.02
G:F d 0–14, g/g	0.60 ^a	0.72 ^b	0.69 ^{bc}	0.69 ^{bc}	0.69 ^{bc}	0.66 ^c	0.03	0.002	0.29	< 0.001

^{a-c}Within a row, means without a common superscript differ ($P < 0.05$).

¹16 pigs were used for each treatment. The least squares means are presented.

²Orthogonal polynomial contrast coefficients were used to determine linear and quadratic effects of increasing ratios of standardized ileal digestible Leu:Lys.

The effects of dietary SID Leu:Lys on PUN and free AA are presented in Table 4. The PUN decreased linearly ($P = 0.02$) by increasing the SID Leu:Lys in the diet with the lowest level observed in pigs fed the 1.00 SID Leu:Lys diet. There was a linear increase in the plasma Leu concentration with an increasing level of SID Leu:Lys in the diet ($P < 0.001$). The plasma Cys concentration increased quadratically ($P < 0.001$), while the plasma concentration of Ile, Lys, Thr, Val, Ala, Glu, Gly, Ser, and Pro decreased quadratically ($P < 0.001$) by increasing SID Leu:Lys in the diet. The plasma concentrations of nondispensable AA, Arg, Met, and Phe were not affected by the SID Leu:Lys content in the diet. The increasing dietary SID Leu:Lys levels did not affect ($P = 0.84$) the overall urea to creatinine ratio of urine (Table 4).

Performance traits were assessed by different descriptive models to estimate the minimum SID Leu:Lys required to obtain the maximum response. The broken-line and curvilinear plateau models were chosen to describe the Leu effect on ADFI and ADG, and the curvilinear plateau model was chosen for G:F (Fig. 1, 2, and 3).

The broken-line models estimated the requirements for the maximum ADFI and ADG at 0.92 ± 0.03 and 0.84 ± 0.01 SID Leu:Lys, respectively. The curvilinear plateau models estimated the maximum ADFI, ADG, and G:F at 1.06 ± 0.09 , 0.94 ± 0.02 , and 0.80 ± 0.01 SID Leu:Lys, respectively. Based on the broken-line models, a Leu supply 10% below the requirement caused a 9% and 16% reduction in ADFI and ADG, respectively. Considering curvilinear plateau models, a 10% decrease in Leu supply below the requirement reduced the ADFI, ADG, and G:F by 2%, 4%, and 10%, respectively.

DISCUSSION

In this experimental design, Lys fulfilled 90% of its requirement, but all the other AA were supplied to meet their requirements in ratio to 100% Lys; therefore, all AA:Lys were exceeding 10% above their requirements. By this design, Leu was limiting until the breakpoint, and Lys became limiting as increments of

Leu were added after breakpoint. At the breakpoint, both Leu and Lys were limiting. Recent studies have shown that the CP level of a cereal-based diet could be reduced to as low as 135 g/kg without compromising performance, as long as the requirements for nondispensable AA are met (Gloaguen et al., 2014). At such a low CP level, soybean meal could be replaced by cereals and crystalline AA (Gloaguen et al., 2014). In commonly used pig diets, Leu deficiency is not a risk, as Leu exists in abundant amounts in most feed ingredients. However, reducing the dietary protein content will reduce Leu content, and it would become a potentially limiting AA. Therefore, in low CP diets, minimum requirements of Leu should be considered due to the important roles of Leu in regulating BCKDH enzyme activity (Wiltafsky et al., 2010), elevating muscle protein synthesis, and inhibiting protein degradation (Murgas Torrazza et al., 2010).

The results of the present study showed greater ADFI and ADG by increasing SID Leu:Lys from 0.70 to 0.90. The fitted models indicated that a minimum supply of 1.06, 0.94, and 0.80 SID Leu:Lys estimated by curvilinear plateau models for ADFI, ADG, and G:F, respectively, was required to support maximum growth performance of pigs from 8 to 12 kg. The estimates for maximum performance by the broken line models were lower than for the curvilinear plateau models (0.92 and 0.84 SID Leu:Lys for ADFI and ADG), as the broken-line model often forces the breakpoint to a lower value (Robbins et al., 2006). According to the variation in animal studies, the animal performance is not a linear response, and therefore the breakpoint values of curvilinear plateau models for ADFI, ADG, and G:F were considered to draw the conclusion, and a minimum SID Leu:Lys of 0.93 was estimated as the minimum requirement to support maximum growth performance of young pigs.

One of the first works on Leu requirement was done by Augspurger and Baker (2004), who tried to estimate Leu requirement for young pigs. However, only in 1 of their 3 experiments could a breakpoint be observed, allowing them to estimate the ideal SID

Table 4. Effect of increasing standardized ileal digestible (SID) Leu:Lys on urine urea to creatinine ratio (U:C), plasma urea nitrogen (PUN), and amino acid concentrations of pigs¹

Item	SID Leu:Lys						SEM	P-value ²		
	0.70	0.80	0.90	1.00	1.10	1.20		ANOVA	Linear	Quadratic
PUN ³ , mmol/L	1.83	1.40	1.38	0.97	1.29	1.07	0.32	0.08	0.02	0.24
U:C ⁴ , mmol/mmol	17.90	16.50	16.69	17.24	19.81	17.99	2.16	0.84	0.47	0.64
Plasma non-dispensable amino acids, mmol/L										
Arg	0.11	0.13	0.10	0.11	0.11	0.11	0.01	0.09	0.51	0.71
Ile	0.52 ^a	0.35 ^b	0.17 ^c	0.12 ^{cd}	0.10 ^d	0.10 ^d	0.03	<0.001	<0.001	<0.001
Lys	0.50 ^a	0.40 ^b	0.33 ^c	0.33 ^c	0.32 ^c	0.36 ^c	0.03	<0.001	<0.001	<0.001
Leu	0.06 ^a	0.09 ^b	0.11 ^{bc}	0.14 ^{cd}	0.16 ^{de}	0.17 ^e	0.01	<0.001	<0.001	0.42
Met	0.11	0.12	0.13	0.12	0.11	0.12	0.01	0.40	0.47	0.32
Phe	0.15	0.16	0.16	0.16	0.14	0.14	0.01	0.30	0.12	0.17
Thr	0.98 ^a	0.57 ^b	0.51 ^{bc}	0.45 ^{bc}	0.38 ^c	0.49 ^{bc}	0.07	<0.001	<0.001	<0.001
Val	1.83 ^a	1.24 ^b	0.59 ^c	0.45 ^{cd}	0.38 ^d	0.40 ^d	0.09	<0.001	<0.001	<0.001
Plasma dispensable amino acids, mmol/L										
Ala	1.13 ^a	1.05 ^a	0.81 ^b	0.83 ^b	0.75 ^b	0.80 ^b	0.05	<0.001	<0.001	0.002
Cys	0.01 ^a	0.02 ^{bc}	0.02 ^{bc}	0.02 ^{bd}	0.02 ^c	0.02 ^d	0.00	<0.001	0.04	<0.001
Glu	0.41 ^a	0.35 ^{ac}	0.22 ^b	0.24 ^{bd}	0.29 ^{cd}	0.26 ^{bd}	0.02	<0.001	<0.001	<0.001
Gly	1.39 ^a	1.10 ^b	0.86 ^c	0.95 ^c	0.89 ^c	0.89 ^c	0.05	<0.001	<0.001	<0.001
Ser	0.40 ^a	0.28 ^b	0.24 ^{bc}	0.23 ^c	0.23 ^c	0.25 ^{bc}	0.03	<0.001	<0.001	<0.001
Pro	0.50 ^a	0.48 ^a	0.41 ^b	0.41 ^b	0.38 ^b	0.42 ^b	0.03	<0.001	<0.001	0.02

^{a-c}Within a row, means without a common superscript differ ($P < 0.05$).

¹Data represent the least squares means of plasma samples from 8 pigs per treatment in 2 wk ($n = 16$). Within a row, means without a common superscript differ ($P < 0.05$).

²Orthogonal polynomial contrast coefficients were used to determine linear and quadratic effects of increasing ratios of standardized ileal digestible leucine to lysine ratio.

³Plasma urea nitrogen.

⁴Urinary urea to creatinine ratio.

Leu:Lys to be close to 1.00 for 10 to 20 kg pigs, which is consistent with the results of the current study.

The estimate of 0.93 SID Leu:Lys by the curvilinear plateau model in the current study is slightly less than the reports of a recent work by Gloaguen et al. (2013a), reporting a minimum 1.02, 1.02, and 0.96 SID Leu:Lys estimated by curvilinear plateau models to maximize ADFI, ADG, and G:F in pigs weighing 10 to 20 kg (Gloaguen et al., 2012). These authors concluded 1.02 SID Leu:Lys as the Leu requirement of young pigs. The 4% decline in ADG in response to a Leu supply 10% below the requirement is also consistent with the 3% decline in ADG reported by Gloaguen et al. (2012).

Previous studies have reported detrimental effects of excess Leu on animal performance (Gatnau et al., 1995, Wiltafsky et al., 2010, Gloaguen et al., 2011). This potential for detrimental effects of excess Leu comes from the interaction among BCAA due to the shared enzymes in their catabolic pathways and the competition of Leu with other BCAA and large neutral AA for transport across the blood-brain barrier that could increase the requirements for Ile, Val (Wiltafsky et al., 2010), and large neutral AA (Smith, 2000; Barea et al., 2009a, 2009b). In comparison to Leu, Val and Ile are re-

ported to have less or no effect on the regulation of the BCKDH complex activity (Cynober and Harris, 2006; Wiltafsky et al., 2009). The results of recently published works from our group showed that Ile may also have a regulatory effect on BCAA catabolism, as an Ile excess decreases performance and plasma Leu concentration (Soumei et al., 2014, Nørgaard et al., 2013).

Valine deficiency causes a rapid decline in animal feed intake, and Leu excess could aggravate the Val deficiency (Gloaguen et al., 2012). However, it appears that a moderate excess of Leu has little or no effect on performance, and a greater excess of Leu could impair both ADFI and ADG in growing pigs. Edmonds and Baker (1987) demonstrated that supplementing 40 g Leu/kg to a basal diet containing 17.4 g Leu/kg did not affect performance in pigs, but supplementing 60 g Leu/kg could impair performance. The detrimental effect of a large excess of Leu also depends on the Ile and Val content of the diet, which contributes to the variation among literature reporting consequences of Leu excess (Edmonds and Baker, 1987, Gatnau et al., 1995, Gloaguen et al., 2011). Gloaguen et al. (2011) also reported that Leu excess can exert growth-depressing effects if Val is deficient. Their results showed that 1.65 SID Leu:Lys but not 1.11 SID Leu:Lys decreased

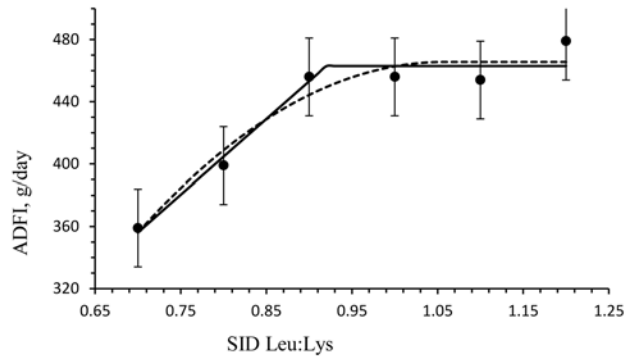


Figure 1. Optimum standardized ileal digestible (SID) Leu:Lys for ADFI determined by a broken-line model was 0.92 ($Y = 463.3 - 485 \times (0.92 - x)$; solid line) and 1.06 by a curvilinear plateau model ($Y = 465.6 - 857.9 \times (1.06 - x)^2$; dashed line). The 95% confidence limits ranged from 0.83 to 1.01 and from 0.76 to 1.35 for the broken-line and curvilinear plateau models, respectively. Data points (·) represent least squares mean \pm SEM for each dietary treatment ($n = 16$ pigs per treatment).

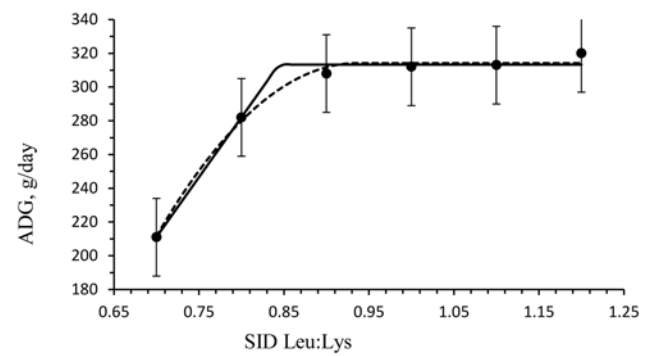


Figure 2. Optimum standardized ileal digestible (SID) Leu:Lys for ADG determined by a broken-line model was 0.84 ($Y = 313.3 - 710 \times (0.84 - x)$; solid line) and 0.94 by a curvilinear plateau model ($Y = 314.2 - 1831.4 \times (0.94 - x)^2$; dashed line). The 95% confidence limits ranged from 0.81 to 0.88 and from 0.87 to 1.01 for the broken-line and curvilinear plateau models, respectively. Data points (·) represent least squares mean \pm SEM for each dietary treatment ($n = 16$ pigs per treatment).

performance when the SID Val:Lys was 0.60 and thus below the requirement, but no effects were observed at 0.70 SID Val:Lys. Wiltafsky et al. (2010) observed that a Leu excess (1.18 vs. 2.11 SID Leu:Lys) impaired growth performance if the SID Leu:Ile ratio exceeded 2.33 or if the SID Leu:Val ratio exceeded 1.79 (Wiltafsky et al., 2010). At the greatest Leu level of 1.20 SID Leu:Lys in the current study, the SID Val:Lys was 0.78, the SID Ile:Lys was 0.59, the SID Leu:Ile was 2.04, and the SID Leu:Val was 1.54. Thus, as long as the Val and Ile requirements are fulfilled in practical diets, there are no indications that Leu causes problems even if provided above the requirement.

The increasing level of the SID Leu:Lys in the diet was accompanied by a clear decline in plasma AA concentrations, resulting in the lowest plasma AA concentrations when the pigs were fed the 0.90 and 1.00 SID Leu:Lys diets. Also, PUN was lowest at the 1.00 SID Leu:Lys, indicating a more efficient AA utilization due

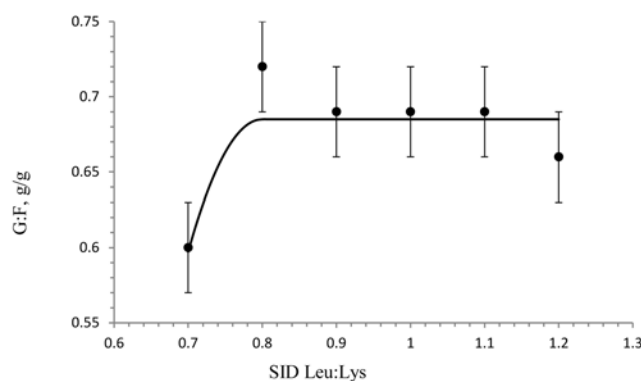


Figure 3. Curvilinear plateau model estimated the optimum standardized ileal digestible (SID) Leu:Lys at 0.80 ± 0.01 ($Y = 0.685 - 8.8 \times (0.80 - x)^2$). The broken-line model could not be fitted to the G:F data. Data points (·) represent least squares mean \pm SEM for each dietary treatment ($n = 16$ pigs per treatment).

to the more balanced AA profile of the diet. The plasma concentration of Ile and Val was reduced to a great extent by increasing SID Leu:Lys in the diet, which was associated with quadratic increases in the plasma Leu concentration. This clearly indicates the role of Leu in regulating the BCKDH enzyme complex activity that seems to be reduced in the Leu deficiency to save the existing Leu from being catabolized, which will result in less catabolism of Ile and Val. By increasing Leu content in the diet, BCKDH activity is being increased, which increases the catabolism of BCAA, mainly through post-transcriptional mechanisms (Wiltafsky et al., 2010). However, supplying Leu above the requirement resulted in further catabolism of Ile and Val, which was reflected in greater PUN and reduced Ile and Val plasma concentrations, although it did not impair ADFI and ADG in this study. This decline in plasma Val and Ile content could not be interpreted as better protein synthesis as long as neither PUN nor the content of the other plasma-free AA were not decreased at the highest Leu level and the decline in Ile and Val concentration therefore originates from their greater catabolism. The α -keto acid of these AA after transamination probably have been used as the energy source. The indications of antagonism between plasma Ile, Val, and Leu are supported by previous studies (Edmonds and Baker, 1987, Wiltafsky et al., 2010).

Conclusions

A minimum of 0.93 SID Leu:Lys is required to support maximum growth performance of young pigs after weaning. This SID Leu:Lys should be considered when diets are formulated with a low CP content. No detrimental effects of Leu above the minimum requirement on animal performance were seen when feeding diets

balanced in AA with no Val or Ile deficiency. Thus, in diets based on commonly used feedstuffs, antagonism among Leu and Ile and Leu and Val is not of practical relevance if the diets fulfill the Ile and Val requirements.

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